PHYSICAL CHEMISTRY 2012

11th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Under the auspices of the
University of Belgrade

Proceedings

The Conference is dedicated to
Professor Ivan Draganić

September 24-28, 2012
Belgrade, Serbia

ISBN 978-86-82475-27-9 ***Volume*** **1**
ISBN 978-86-82475-28-6 ***Volume*** **II**

Title: PHYSICAL CHEMISTRY 2012 (Proceedings)
Editors: S. Anić and Ž. Čupić
Published by: Society of Physical Chemists of Serbia, Studenski trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan" Priting and Publishing Company; 200 Copies;
Number of pages: 6+ 497; **Format**: B5; Printing finished in September 2012.

Text and Layout: "Jovan"

200- *Coppy printing*

CONTENTS

F-06-P

NITRIC OXIDE AND PROTEIN CARBONYL CONTENT IN THE LIVER OF STRESSED RATS

J. Zlatković, M. Demajo, D.Filipović

Laboratory for Molecular Biology and Endocrinology, VINČA Institute of Nuclear Sciences, University of Belgrade, P.O.Box 522, Belgrade, Serbia

Abstract

Nitric oxide (NO) has been identified as a source of oxidative/nitrosative stress that is known to oxidatively modify DNA, lipids and proteins. One such modification is the addition of carbonyl groups to amino acid residues in proteins. Therefore, the aim of our study was to examine the effects of acute, chronic or combined stress on NO production and protein carbonyl content in the cytosolic fraction of rat liver. Since NO is a highly reactive molecule, the levels of NO metabolites (nitrates and nitrites) as markers of stable end products of NO metabolism were measured. Both acute stresses showed unchanged nitrite levels while only acute IM stress led to an increased level of the carbonyl group. The NO metabolites and protein carbonyl content were increased by chronic isolation and remained upregulated after combined stress. These data indicate that chronic isolation stress with increased NO metabolites led to nitrosative stress, whereby accumulation of oxidized proteins in the liver may induce progressive liver damage.

Introduction

It has been shown that oxidative/nitrosative stress has been implicated as a contributor to liver injury. Moreover, it may derange nitrogen metabolism in hepatocytes where nitric oxide (NO) is involved among the other factors regulating this metabolic pathway. NO is an inorganic reactive nitrogen species synthesized in the liver by inducible nitricoxide synthase found in hepatocytes, Kupffer cells, and endothelial cells, or by endothelial nitric oxide synthase. NO and superoxide react spontaneously to form the potent and versatile oxidant, peroxynitrite. This highly toxic species reacts with lipids, proteins and DNA. Our laboratory has previously demonstrated that chronic isolation stress causes oxidative stress in rat liver, evidenced by compromised CuZnSOD and MnSOD activity [1]. To further elucidate the role of nitrosative stress in the liver, we investigated the effects of acute, chronic or combined stress on NO production. Moreover, the formation of carbonyl derivatives of amino acids was used as an index of protein oxidation associated with oxidative stress.

Experimental

The male Wistar rats (body weight 330–400 g) were divided into four groups: control (Con); acute stress [2 h of immobilization (IM) or cold (C) stress (at 4°C)];

chronic isolation (IS) [individual housing of rats for 3 weeks]; combined stressors (IS+IM, IS+C) i.e. rats undergo IS stress followed by a single exposure to 2 h of either IM or C stress. Nitric oxide production in the cytosolic fraction of liver was quantified by measuring nitrate/nitrite, by the Griess method [2]. Liver protein carbonyl content was determined by the colorimetric method. Briefly, protein samples containing 6 mg/ml of protein were resuspended in 10 mM 2, 4-dinitrophenylhydrazine (DNPH) in 2M HCl and incubated for 60 min. The samples that had been previously precipitated with trichloroacetic acid (20%), centrifuged at 11, 000 × g were washed three times with ethanol-ethyl acetate (1:1; v/v) to remove the residual DNPH reagent. The final precipitates were dissolved in 6 N guanidine hydrochlorid and protein carbonyl content was determined by measuring the absorbance at 375 nm, using a molar absorption coefficient of 22, 000 $M^{-1}cm^{-1}$. Liver cytosolic protein concentration was measured by the Lowry method. Data were analyzed by two-way ANOVA followed by Duncan post hoc test.

Results and discussion

Data presented in Fig. 1 and 2. show NO metabolites (nitrate/nitrite) content and carbonyl content in the cytosolic liver fraction of rat exposed to acute, chronic or combined stress, respectively.

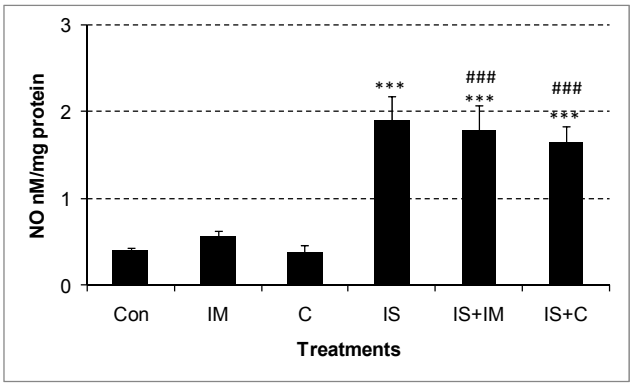

Figure 1. Effect of acute (immobilization IM or cold C) stress, chronic isolation (IS) or their combination (IS+IM and IS+C) on hepatic nitrite/nitrate levels. Values are mean ± SEM of 6 animals for each group. ***Compared to control group ($p < 0.001$); combined stress *vs* acute stress ($^{###}p < 0.001$).

Two-way ANOVA analysis revealed a significant effect of chronic stress ($F_{1.30}$ = 78.72, $p<0.001$) on nitrate content. Both acute IM and C stress did not alter nitrite content when compared to the control ($p>0.05$). Duncan post hoc test showed a significant increase of nitrite content in chronic IS stress and both combined IS+IM and IS+C stress ($p<0.001$). Moreover, significant increases of nitrite content of both combined IS+IM and IS+C stress compared to acute stress alone were found ($^{###}p<0.001$). Increased NO end-product levels in the liver, following chronic IS and combined stress, indicate a state of nitrosative stress which may be responsible for liver damage.

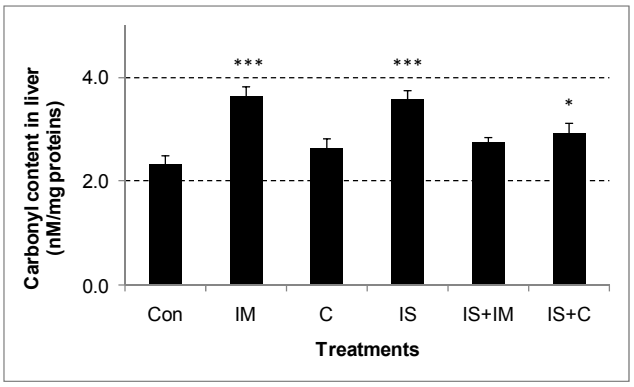

Figure 2. Effect of acute (immobilization IM or cold C) stress, chronic isolation (IS) or their combination (IS+IM and IS+C) on protein carbonyl levels. Values are mean ± SEM of 6 animals for each group. Asterisk indicates comparison between stressed groups and control group (*$p < 0.05$; ***$p < 0.001$).

The hepatic protein carbonyl content values ranged from 2.14 to 2.50 nM/mg of protein in control rats. Opposite to acute C stress, significant increase in protein carbonyl content was observed following acute IM stress ($p<0.001$). Moreover, significant increases of carbonyl content following chronic IS and combined IS+C stress were also measured ($p<0.001$, $p<0.05$ respectively). The previous reported compromised antioxidant defense mechanisms after chronic IS stress, evidenced by decreased hepatic CuZnSOD and MnSOD activities [1], resulted in increased protein oxidation as indicated by increased protein carbonyl. This suggests that the accumulation of oxidized proteins in the liver may be an early indication of chronic IS stress-induced liver damage.

Conclusions

Chronic IS stress concomitantly increases NO metabolites (nitrate and nitrite) and protein carbonyl levels. Increased levels of NO metabolites, which may be due to an increase in the NO synthase activity, indicate a nitrosative state. Increased protein carbonyl groups suggest that these oxidized species may be useful as diagnostic biomarkers for progressive liver damage.

Acknowledgement

This work was supported by the Ministry of Education and Science of the Republic of Serbia, Grant 173023.

References

[1] J. Zlatković, D. Filipović, Mol Cell Biochem., 2011, 357, 143-150.
[2] J. A. Navarro-Gonzálvez, C. García-Benayas, J. Arenas. Clin. Chem., 1998, 44, 679-681.